Geosmin causes off-flavour in arctic charr in recirculating aquaculture systems

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Abstract

The ‘earthy’ and ‘muddy’ off-flavours in pond-reared fish are due to the presence of geosmin or 2-methylisoborneol in the flesh of the fish. Similar off-flavours have been reported in fish raised in recirculating aquaculture systems (RAS); however, little information is available regarding the cause of these off-flavours. Our hypothesis was that earthy and muddy off-flavour compounds, found previously in pond-raised fish, are also responsible for off-flavours in fish raised in RAS. In this preliminary study, we examined water, biofilms in RAS and fillets from cultured arctic charr known to have off-flavours and requiring depuration using instrumental [solid-phase microextraction procedure and gas chromatograph-mass spectrometry (GC-MS)] and human sensory analyses. Geosmin was present in the samples taken from the biofilter and on the side walls of the tanks. Two-methylisoborneol was only found in low levels in the samples. The GC-MS results indicated the presence of geosmin in the fillets (705 ng kg⁻¹), but lower levels were found in the water (30.5 ng L⁻¹). Sensory analyses also detected an earthy flavour (i.e., geosmin presence) in the fillets, and, therefore, it appears that geosmin is the main compound responsible for the off-flavour in RAS. Further studies are being performed to identify the microorganisms responsible for geosmin production in RAS.

Keywords: geosmin, off-flavour, arctic charr, recirculating aquaculture systems

Introduction

‘Off-flavour’ in aquacultured products is a problem that can be costly for the industry and can also lead to the loss of sales. The problem was first recognized in farmed fish raised in outdoor ponds but it is now also being observed in fish raised in recirculating and partial recirculating systems (Schrader & Dennis 2005; Robin, Cravedi, Hillenweck, Deshayes & Vallod 2006). The off-flavours most frequently encountered are ‘earthy’ and ‘muddy’. The earthy and muddy flavours in cultured fish are due to the presence of geosmin or 2-methylisoborneol (MIB) in the flesh of the fish (Tucker 2000; Howgate 2004). These compounds are semivolatiles and, when present in the water, are absorbed by the fish and stored in lipid-rich tissues. Planktonic and benthic cyanobacteria and actinomycetes are known producers of MIB and geosmin (Grimm, Lloyd, Zimba & Palmer 1999). The process of elimination of these compounds by the fish is much longer than the absorption. The elimination rate decreases with lower temperature water and is slower in fish with more fatty tissues (Tucker 2000). In a commercial operation, a depuration time is required before the harvesting in order to gradually
eliminate the earthy-muddy taste produced by geosmin and MIB. Most off-flavour-related research has been performed with channel catfish raised in USA (Tucker 2000) and relatively little research has been performed with other types of fish, such as char, raised in recirculating systems. This is the first report on the issue of off-flavour aimed at Arctic charr (Salvelinus alpinus) largely raised in recirculating aquaculture systems (RAS) as it displays clear adaptability to crowding, tolerance to disease and fetches a high market price (Le François, Lemieux & Blier 2002). Our hypothesis was that the compounds involved in the production of off-flavours in pond-raised fish are responsible for off-flavours encountered in RAS. In this primary study, we analysed fillets from cultured Arctic charr that were off-flavour and subsequently required depuration before they could be sold to processors. Instrumental and human sensory analyses were used to identify the types of off-flavour and the compound(s) responsible for the earthy off-flavour in the fillets.

Materials and methods

Sample collection and fillet preparation

Arctic charr were from a commercial farm operated in Man, WV, USA. The charr were cultured indoor in dual-drain fibreglass circular tanks (640 m$^3$) under dim light. The water temperature was maintained at 12.6–13.0°C. The Arctic charr densities in the grow-out tanks were between 120 and 140 kg m$^{-3}$. The production system was a water-recirculation unit (95% water exchange) that included biofiltration and pure oxygen injection. Fish were fed industrial manufactured pellets from Zeigler (Gardners, PA, USA), using an automatic feeder for 16 h a day at 10–12-min intervals and contained 45% protein and 20% fat. Ten fish were collected from four tanks (10 fish x four tanks); two grow-out tanks and two depuration tanks, 3 and 7 days of depuration. The depuration system included a sand filtration and aeration. Collected fish were euthanized by farm personnel with a sharp blow to the head immediately filleted and the skin was also removed. Two fillets per fish were obtained and placed in separate plastic (Ziploc, SC Johnson Canada, Brantford, ON, Canada) bags. The fillets were then frozen until they were shipped overnight for the off-flavour analysis to the United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit (NPURU) at the Thad Cochran Research Center in University, Mississippi.

Sample collection of water and biofilms

Six water samples were taken in each tank; the samples were taken from different places in the tank. Six biofilm samples were also taken from each tank. Individual water samples were placed in 20 mL glass scintillation vials with foil-lined caps (Fisher Scientific, Ottawa, ON, Canada). The vials were filled completely so that no air bubbles were observed when the vial was capped and inverted. Individual biofilm samples were also placed in 20 mL glass scintillation vials. Biofilm material was scraped from the side of the tanks and the biofilm was then placed into the vials. The samples were refrigerated (4°C) until shipping to the United States Department of Agriculture, Agricultural Research Service, NPURU at the Thad Cochran Research Center in University, Mississippi.

Analysis of fillets by microwave distillation and solid-phase microextraction-gas chromatograph-mass spectrometer (SPME-GC-MS)

The method of Schrader, Dhammika Nanayakkara, Tucker, Rimando, Ganzera and Schaneberg (2003) as modified from the method of Lloyd, Lea, Zimba and Grimm (1998) was used to analyse geosmin and MIB from fillets. For each fillet of Arctic charr, 20 ± 0.5 g of fish were weighed into a glass distillation flask. The flask was then heated in the microwave (General Electric, New York, NY, USA; model JES1036WF0001) for 5 min at power level ’3’ while purging with 80 mL L$^{-1}$ of N$_2$ gas. The collected distillate was cooled in a water and antifreeze bath (Fisher Scientific, model Isotemp 3006) and the volume was adjusted to 20 mL using nanopure water. From each 20 mL of sample, aliquots of 600 μL were placed into 2 mL glass vials containing 0.3 g of NaCl. Each vial was sealed with a crimp cap. These vials were stored at 4°C until ready for analysis by SPME-GC-MS using the same method as that described for the analysis of water samples.

Sensory determination of fillet taste and aroma

The fillets (40 in total) were sent to the Garrison Sensory Evaluation Laboratory at Mississippi State University (Starkville, MS, USA) for sensory analysis by a trained panel. The samples were removed from the
freezer and immediately wrapped and sealed in an aluminium foil. The samples were prepared and cooked using AOAC method 976.16 for baking in foil. They were specifically baked at 190.56 °C in a pre-heated oven to an internal temperature of 71.11 °C. After the samples were cooked, a small amount of the fillet was placed in a Teflon™ (Dupont, Wilmington, DE, USA) sniff bottle. The remainder of the fillet was left in the aluminium foil. The samples were presented to a group of six panellists, and the aroma and taste were recorded on a 10 cm hedonic scale.

Analysis of geosmin and MIB levels in water and biofilm samples

The analyses were performed at the NPU, Thad Cochran Research Center in University, Mississippi. The method used to quantify the levels of geosmin and MIB utilized the SPME procedure adapted from Lloyd et al. (1998). Aliquots of 600 μL were pipetted individually into 2 mL glass screw-top vials with sodium chloride at the bottom (0.3 g vial⁻¹). The vials were heated at 40 °C for 20 min before the volatile compounds were adsorbed onto a 100 μm polydimethylsiloxane SPME fibre (Supelco, Bellfonte, PA, USA). The fibre assembly was then shaken for 10 min during the absorption period and desorbed for 2 min at 250 °C in the injection port of an HP 6890 GC-MS (Agilent, Palo Alto, CA, USA) operated in the selected ion monitoring mode. The conditions of the gas chromatogram were as follows: (1) the initial oven temperature was 60 °C for 0.5 min; (2) followed by a ramp rate of 30 °C min⁻¹ to 100 °C; (3) followed by a ramp rate of 20 °C min⁻¹ to 300 °C with an isothermal time of 2 min; and (4) the maintenance of flow pressure was at 18 lb in⁻² with helium used as a carrier gas. The molecular ion base peaks were monitored at m/z 168, 95 and 135 for MIB and at m/z 182, 112 and 126 for geosmin. The capillary column used was a DB-5 (5%-phenyl-methylsiloxane, 30 m, 0.25 mm inside diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). The retention time was 6.8 min for geosmin and 5.2 min for MIB. Standards for MIB and geosmin were prepared at 0.1, 0.5, 1.0 and 2.5 μg L⁻¹ in deionized water. These standards were obtained from Wako Chemicals USA (Richmond, VA, USA) and were included at the beginning, middle and end of each group of samples analysed using a CombiPal Autosampler (Leap Technologies, Carrboro, NC, USA). Each sample was run in triplicate (Schrader et al. 2003).

Isolation of microorganisms in biofilm samples

The analyses were performed at the NPU, Thad Cochran Research Center in University, Mississippi. Biofilm samples were inoculated into different media (ASM, BG-11, Allen, BG-11 and J) contained in 6 well plates and incubated for 2 weeks at 28 °C under cool-white fluorescent lights. After 2 weeks, a sample from each well was centrifuged to concentrate the algae at the bottom of the centrifuge tube and observed under a phase-contrast microscope to determine the presence of cyanobacteria. When present, the cyanobacteria were isolated using the manual direct isolation technique. For isolation, the sample was observed using a dissecting stereoscope (Olympus SZH10, Center Valley, PA, USA) and one filament of the cyanobacteria was removed with a capillary tube that had been separated in half over a Bunsen burner flame. The filament was then placed in a well containing BG-11 at 28 °C for a week. The isolated filaments were then observed under the microscope to determine whether isolation had been achieved. Isolated cyanobacteria were identified using the reference book Fresh Algae of North America: Ecology and Classification (Wehr & Sheath 2003). The isolated cyanobacterial cultures were also tested for the production of geosmin and MIB using SPME-GC-MS.

Isolation of actinomycetes

Aliquots (0.1 mL) of water and biofilm samples were spread-plated on yeast–dextrose agar and also on actinomycete isolation agar plates. The plates were then incubated at different temperatures: 14, 20 and 28 °C. The plates were inspected after 2 days and at 7 days of incubation for the presence of actinomycete colonies. Suspected colonies were transferred to fresh agar plates and restreaked to confirm isolation. These ‘isolation’ plates were inspected after 2 and 7 days of incubation for earthy or musty odour production.

Statistical analysis

The tanks were used as the experimental units (n = 4). Partially hierarchical analysis of variance with three factors [tank, treatment (grow-out or depuration) and the location of measure (fillet, water or biofilm)] (split-plot with replication) was performed to detect differences between treatments, followed by Tukey's multiple comparison test. The tank
was nested within treatment. The equality of variance of residuals was analysed using Levene’s test and normality of residuals using the Kolmogorov–Smirnov test. To reach normality, MIB data were log transformed and geosmin data were ranked following the general rank approach (Conover & Iman 1981). When no differences were found between the tanks and the treatment, linear regression analyses were used to determine the correlation between the geosmin levels in the fillets and in the water and also between the geosmin levels in the fillets and the earthy-flavour intensity of the fish. All statistical tests were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA) and SYSTAT version 12 (Systat Software, Chicago, IL, USA).

Results and discussion

The GC-MS results indicated the presence of geosmin in the fillets, water and biofilm samples (Table 1). Geosmin concentrations were higher in the samples taken from the biofilm on the side walls of the tanks and in the Arctic charr fillets (biofilm: 381 ± 634 and 348 ± 393 ng kg⁻¹ in grow-out and depuration tanks respectively; fillet: 703 ± 493 and 706 ± 619 ng kg⁻¹ in grow-out and depuration tanks respectively; Table 1). Two-methylisoborneol was present in ‘trace’ amounts in the fillets (8 ± 8 and 2 ± 2 ng kg⁻¹ in grow-out and depuration tanks respectively; Table 1) compared with geosmin levels. An ANOVA indicated that the values did not differ significantly between the treatments ($F_{1,4} = 0.123; P = 0.799$) or the tanks ($F_{1,4} = 0.377; P = 0.708$) for the geosmin levels. The same results were obtained for MIB (treatments: $F_{1,4} = 0.833; P = 0.458$; tanks: $F_{1,4} = 1.166; P = 0.399$). The MIB does not appear to be the compound responsible for the off-flavour in the charr, because the levels found in this study are well below the detectable limit in rainbow trout (600 ng kg⁻¹) (Tucker 2000). The detectable limit for the geosmin in rainbow trout fillet is 900 ng kg⁻¹ (Robertson, Jauncey, Beveridge & Lawton 2005). The geosmin levels in the fish fillet from the grow-out tanks and the depuration tanks are not significantly different, likely as a result that the fish in the depuration tanks had only been depurated for 3 and 7 days. Depuration requires more time for fatty fish such as charr, and when held at a low temperature (Tucker 2000). Therefore, geosmin appeared to be the main compound responsible for the ‘earthy’ off-flavour in the fillet.

Linear regression analysis confirmed a correlation (correlation coefficient $r^2 = 0.88$) between geosmin levels in the water and the geosmin levels in the fillets, although not significant ($R = 0.776$, $P = 0.224$; Fig. 1). We acknowledge that the number of samples used for the calculation of the linear regression is low ($n = 4$) but our approach involved working in a commercial operation environment (limited access to tanks) in order to provide the presence or absence of MIB and geosmin in conjunction with off-flavour expression in cultivated fish in intensive recirculation systems, i.e. RAS. The fact that geosmin levels in the water and fish are not highly correlated is not surprising. If cyanobacteria are the source of the geosmin, the timing of cyanobacterial synthesis of geosmin, release of geosmin in the tank water from senescing cyanobacterial cells, the uptake by the fish and the depuration time were probably not synchronized (Grimm & Zimba 2003). The depuration of

![Figure 1](image-url)  
**Figure 1** Linear regression comparing geosmin levels in water and Arctic charr, $r = 0.77$.

### Table 1 Mean values of geosmin and MIB levels present in the fillets, water and biofilms from four tanks: two grow-out tanks and two depuration tanks, 3 and 7 days of depuration

<table>
<thead>
<tr>
<th>Tanks</th>
<th>Fillet</th>
<th>Water</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geosmin (ng kg⁻¹)</td>
<td>MIB (ng kg⁻¹)</td>
<td>Geosmin (ng kg⁻¹)</td>
</tr>
<tr>
<td>Grow-out</td>
<td>703 ± 493</td>
<td>8 ± 8</td>
<td>37 ± 17</td>
</tr>
<tr>
<td>Depuration</td>
<td>706 ± 619</td>
<td>2 ± 2</td>
<td>24 ± 17</td>
</tr>
</tbody>
</table>

MIB, 2-methylisoborneol.
earthy and musty off-flavour compounds by fish is slow compared with their uptake (Tucker 2000). If the main route for geosmin accumulation in the charr flesh is via passive diffusion across the gills from the water, bioconcentration of geosmin in the flesh is occurring because the geosmin levels in the water were significantly lower than those detected in the fish flesh. However, until the source of geosmin in the RAS is identified, geosmin tank-fish dynamics cannot be further discussed here.

The fillets were also analysed by a sensory panel to detect and rate the intensity of all types of off-flavours. Each of the six panellists rated the fillets on a hedonic scale of 1.2–9.4 (no off-flavour taste = 1.2; 9.4 = strongest off-flavour), and the scores were averaged (Table 2). The off-flavour that the panellists were expected to detect was earthy because the presence of geosmin had previously been verified instrumentally. The panellists also detected other off-flavours in the charr that could be influencing the taste and aroma values (Table 3). The sensory data related to earthy-muddy intensity were analysed by linear regression to determine whether it showed a relationship with the instrumental analytical results. The coefficient of determination ($r$) was 0.96 and indicated a significant relation ($P = 0.036$) between the sensory results and the instrumental results for the presence of geosmin (Fig. 2). Conclusively, the sensory analysis helped confirm that geosmin was responsible for the earthy off-flavour in the Arctic charr.

**Table 2** Mean values of the sensory results for earthy off-flavour in the Arctic charr fillets

<table>
<thead>
<tr>
<th>Tanks</th>
<th>Earthy (sensory rating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tank 1 (grow-out)</td>
<td>6.916</td>
</tr>
<tr>
<td>Tank 2 (grow-out)</td>
<td>4.956</td>
</tr>
<tr>
<td>D1 (depuration 3 days)</td>
<td>7.976</td>
</tr>
<tr>
<td>D2 (depuration 7 days)</td>
<td>5.096</td>
</tr>
</tbody>
</table>

**Table 3** Other off-flavours detected by the trained sensory panellists

<table>
<thead>
<tr>
<th>Off-flavour</th>
<th>Number of fillets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metallic</td>
<td>12</td>
</tr>
<tr>
<td>Bitter</td>
<td>3</td>
</tr>
<tr>
<td>Amoniac</td>
<td>2</td>
</tr>
<tr>
<td>Cat box</td>
<td>1</td>
</tr>
<tr>
<td>Umami</td>
<td>1</td>
</tr>
<tr>
<td>Mud</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 2 Linear regression comparing geosmin levels in Arctic charr with the sensory analysis (rating), $R = 0.96$.

Planktonic cyanobacteria species are commonly attributed as the causes for the musty and earthy off-flavours in commercial channel catfish (*Ictalurus punctatus*) cultured in ponds in the southeastern USA, and examples include *Oscillatoria perornata*, a producer of MIB, and *Anabaena* spp., producers of geosmin (Tucker 2000). In our study, these genera of cyanobacteria were not observed in the water and biofilm samples. In addition, several other cyanobacteria species isolated from the biofilm samples were determined not to produce geosmin. However, a cyanobacterium identified to be a species of *Pseudanabaena* (isolated from a biofilm sample) was confirmed to be a MIB-producer. Because the main off-flavour in the charr was earthy and identified to be due to the presence of geosmin in the fish flesh, this *Pseudanabaena* sp. is not considered to be a contributor to the earthy off-flavour problem. However, the isolation of an MIB-producing species of cyanobacteria from the recirculating system provides evidence for the potential existence of other odour-producing cyanobacteria as *in situ* sources for other types of off-flavours in similar types of indoor culture systems. No odour-producing actinomycetes were detected in any of the water or the biofilm samples, and so they are also not believed to be the primary cause of the off-flavour problem. At this time, the main producer of geosmin in these tanks is still unknown, but we believe certain species of cyanobacteria that were abundant in the biofilm layers on the inside of the tanks to be the likely source.

Our attempts to isolate a geosmin-producing species microorganism have relied on conventional cultivation techniques so far. The inability to isolate a geosmin-producing bacterial species can be attributed to one of the following: (1) the culture media use was inappropriate for culturing the particular geos-
min-producing microorganism or (2) the presence of a geosmin-producing bacterial species in the biofilm and possibly the biofilter that entered a viable but nonculturable state. Future studies may include the use of specific oligonucleotide probes (e.g., 16S rRNA) to help characterize and identify potential geosmin-producing bacteria in the biofilm and RAS tank-wall biofilms (Kalmbach, Manz & Szewzyk 1997).

Conclusions
This research is the first report identifying an earthy off-flavour in Arctic charr cultured in RAS. In our study, we confirmed the presence of geosmin in the fish flesh as the cause of the earthy off-flavour in the charr. Although the sources of geosmin in the RAS were not identified, preliminary evidence indicates microorganisms found in the biofilm layers on the tank walls to be one potential source.

Acknowledgments
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References


